UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: June 23, 2016

Subject: Efficacy Review for Frozen
EPA File Symbol 5813-RRE, DP Barcode: 433652

From: Alison Clune
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Antimicrobials Division (7510P)

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Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: The Clorox Company
c/o PS&RC
P.O. Box 493
Pleasanton, CA 94566-0803

Formulation from the Label:

Active Ingredient	% by wt.
Sodium dichloroisocyanurate dihydrate	14.85%
Other Ingredients	85.15%
Total	100.00%

I BACKGROUND

The product, Frozen (EPA Reg. No. 5813-RRE) is a new product submitted for registration as a hard surface disinfectant (bactericide, virucide, and fungicide) and non-food contact surface sanitizer for use on hard, non-porous surfaces in residential and commercial settings. The studies were conducted at Accuratus Lab Services, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 and MicroBioTest (Division of Microbac Laboratories, Inc.), 105

Carpenter Drive, Sterling, VA 20164.

This data package contained a letter from the applicant's agent to EPA (dated February 22, 2016), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), 14 new efficacy studies (MRID Nos. 49741206 through 49741219), the Confidential Statement of Formula (CSF) (one Basic formulation), and the proposed label (dated 2/18/16). Statements of No Data Confidentiality Claims, Good Laboratory Practices, Quality Assurance, and certificates of analysis of the active ingredient in each product batch were included for each study.

II USE DIRECTIONS

The product is for disinfecting and sanitizing hard, non-porous surfaces. The product may be used to treat hard, non-porous surfaces such as appliance exteriors, bathtubs, cat litter boxes, counter tops, diaper pails, glass, garbage cans, glazed porcelain, plastic laminate, plastic patio furniture, showers, sinks, steering wheels, trash compactors, and walls. The label indicates the product may be used on glass, glazed ceramic tile, glazed porcelain, linoleum, plastic (e.g., vinyl) and washable walls.

Directions on the proposed label provide the following information regarding preparation and use of the product:

To disinfect Hard, Nonporous Surfaces:

Fill dosing cap to Line 1 (2 Tbsp). Add crystals to 1 gallon of water. Stir to dissolve. Mop or wipe surface with bleach solution. Allow solution to contact surface for 10 minutes. For highly soiled surfaces, precleaning is required.

To sanitize:

Fill dosing cap to line 1 (2Tbsp). Add crystals to one gallon of water. Stir to dissolve. Mop or wipe surface with bleach solution. Allow solution to contact surface for 5 minutes. For highly soiled surfaces, precleaning is required.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces against a Broad Spectrum of Bacteria:
The effectiveness of disinfectants for use on hard surfaces must be substantiated by data derived using the AOAC Use-Dilution Method or the AOAC Hard Surface Carrier Test for water soluble powders and liquid products, or the AOAC Germicidal Spray Products as Disinfectants Method for spray products and towelettes. Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, tested at or below the lower certified limit of the active ingredient(s) against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). The 3 product lots must be tested against *Staphylococcus aureus* on 3 separate days. To support products labeled as "general disinfectants" for the AOAC Use-Dilution Method, killing on 59 out of 60 carriers for *Salmonella enterica* and 57 out of 60 carriers for *Staphylococcus aureus* is required to provide effectiveness at the 95% confidence level. For the AOAC Germicidal Spray Products as Disinfectants Method, killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level and a mean log density of 5.0-6.0

logs for *Salmonella enterica* and at least 6.0 logs for *Staphylococcus aureus* must be achieved. For the AOAC Hard Surface Carrier test, killing on 58 out of 60 carriers is required to provide effectiveness at the 95% confidence level and the dried carrier count is to be 0.5 – 2.0 X 10^6 for *Salmonella enterica* and 1 – 5 X 10^6 for *Staphylococcus aureus*.

Disinfectants for Use on Hard Surfaces against a Broad Spectrum of Bacteria (Additional Bacteria):

The effectiveness of disinfectants for use on hard surfaces against additional bacteria must be substantiated by data derived using the AOAC Use-Dilution Method or the AOAC Hard Surface Carrier Test for water soluble powders and liquid products, or the AOAC Germicidal Spray Products as Disinfectants Method for spray products and towelettes. Ten carriers should be tested against each specific bacterium for each of two samples representing two different batches tested at or below the nominal concentration of the active ingredient(s). The product should kill all the test microorganisms on all carriers in less than 10 minutes. The minimum carrier count to make the test valid should be 1 x 10^4.

Virucides:

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. The Agency recommends the use of ASTM E1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces. Carrier methods that are modifications of the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants and towelettes) may be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. If the product is intended to be represented as a one-step virucidal, an appropriate organic soil (i.e. 5% blood serum) should be included with the viral inoculum.

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The agency recommends using the ASTM Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces (E1153). Tests should be performed with each of 3 product samples, representing 3 different product lots, tested at or below the lower certified limits of the active ingredients against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048). The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous, using 5 test carriers and 3 control carriers. The ASTM method states that the inoculum employed should provide a count of at least 7.5 x 10^5 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9% over the parallel control within 5 minutes.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

When claims are made for the effectiveness of the product in hard water, all data should be developed at the hard water tolerance claimed. The hard water tolerance level may differ with the level of antimicrobial activity claimed.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. **MRID 49741206 "AOAC Use-Dilution Method, Test Organism: *Staphylococcus aureus* (ATCC 6538)" for Frozen, F2015.0012, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number A18315.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC), Batch No. 15HLD02 was reported to be **14.12%** SDIC, and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three batches (Batch Nos. 15HLD01, 15HLD02, and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.2 (copy provided). The product was received as a granulated solid. A use solution was prepared for Batch 15HDL01 by dissolving 6.00 g of test substance in 810.8 mL of 100 ppm AOAC synthetic hard water. For batches 15HDL02 and 15HDL03, the use solution was made by dissolving 11.10 g of test substance in 1500 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. An SDIC titration was performed on the prepared test substance. A 10 µL aliquot of a thawed, vortex mixed cryovial of stock organism broth culture was transferred to an initial 10 mL tube of growth medium (Synthetic broth), mixed and incubated for 24 ± 2 hours at 35-37°C. Aliquots of 10 µL of the 24 hour culture were transferred to a sufficient number of tubes containing 10 mL culture medium. Daily transfers were performed once for testing on 5/4/15, twice for testing on 5/5/15, and four times for testing on 5/8/15. The final test culture was incubated for 48-54 hours at 35-37°C. The final culture was vortex mixed (3-4 seconds) and allowed to stand for ≥ 10 minutes before use. For testing on 5/4/15 and 5/5/15, 40.0 mL of the culture was diluted with 40.0 mL sterile growth medium. For testing on 5/8/15, 36.0 mL of the culture was diluted with 36.0 mL sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinders per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 ± 2 minutes. The carriers were dried for 38 minutes at 36.0-36.2°C at 42-55.8% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 19.0-21.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Representative test and positive control subculture tubes showing growth were subcultured to Tryptic Soy Agar with 5% sheep's blood and

incubated at 35-37°C for one day. The resultant growth was examined, Gram stained, and biochemically assayed to confirm or rule out the presence of test organism. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: No protocol amendments occurred during the study. A protocol deviation occurred when a 4.86% organic soil load was inadvertently prepared on 4/30/15 for testing of Batch 15HLD01. Testing was repeated for Batch 15HLD01 on 5/8/15.

2. **MRID 49741207 "AOAC Use-Dilution Method, Test Organism: *Pseudomonas aeruginosa* (ATCC 15442)" for Frozen, F2015.0012, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number A18316.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC), Batch No. 15HLD02 was reported to be **14.12%** SDIC, and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three batches (Batch Nos. 15HLD01, 15HLD02, and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.3 (copy provided). The product was received as a granulated solid. A use solution was prepared for Batch 15HDL02 by dissolving 11.10 g of test substance in 1500 mL of 100 ppm AOAC synthetic hard water. For batches 15HDL01 and 15HDL03, the use solution was made by dissolving 7.40 g of test substance in 1000 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. An SDIC titration was performed on the prepared test substance. A 10 µL aliquot of a thawed, vortex mixed cryovial of stock organism broth culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated for 24 ± 2 hours at 35-37°C. Aliquots of 10 µL of the 24 hour culture were transferred to a sufficient number of tubes containing 10 mL culture medium. Three additional daily were performed once for testing on 5/4/15 and twice for testing on 5/6/15 and 5/20/15. The final test culture was incubated for 48-54 hours at 35-37°C, and the pellicle was removed via vacuum aspiration. The culture was then vortex mixed (3-4 seconds) and allowed to stand for ≥ 10 minutes before use. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. For testing on 5/4/15 and 5/6/15, carriers were dried for 38 minutes at 36.0-36.3°C at 42-55.7% relative humidity. For testing on 5/20/15, carriers were dried for 41 minutes at 35.0-36.1°C at 54.9% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 20.0-20.5°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Representative test and positive control subculture tubes showing growth were subcultured to Tryptic Soy Agar with 5% sheep's blood and incubated at 35-37°C for one day. The resultant growth was examined, Gram stained, and biochemically assayed to confirm or rule out the presence of test organism. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: No protocol amendments occurred during the study. A protocol deviation occurred when a 4.86% organic soil load was inadvertently prepared on 4/30/15 for testing of Batch 15HLD01. Testing was repeated for Batch 15HLD01 on 5/6/15. Due to a carrier control failure on 5/5/15, testing of Batch 15HLD03 was repeated on 5/20/15.

3. **MRID 49741208 "AOAC Use-Dilution Method, Test Organism: *Salmonella enterica* (ATCC 10708)" for Frozen, F2015.0012, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number A18314.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC), Batch No. 15HLD02 was reported to be **14.12%** SDIC, and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Salmonella enterica* (ATCC 10708). Three batches (Batch Nos. 15HLD01, 15HLD02, and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.1 (copy provided). The product was received as a granulated solid. A use solution was prepared for Batch 15HDL01 by dissolving 18.50 g of test substance in 2500 mL of 100 ppm AOAC synthetic hard water. For batches 15HDL02 and 15HDL03, the use solution was made by dissolving 7.41 g of test substance in 1000 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. An SDIC titration was performed on the prepared test substance. A 10 µL aliquot of a thawed, vortex mixed cryovial of stock organism broth culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated for 24 ± 2 hours at 35-37°C. Three additional daily transfers were performed. The final test culture was incubated for 48-54 hours at 35-37°C, and was vortex mixed (3-4 seconds) and allowed to stand for ≥ 10 minutes before use. The test organism was diluted by adding 14.0 mL of test organism suspension to 196.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. The carriers were dried for 38 minutes at 36.1-36.3°C at 53.1% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: No protocol amendments or deviations occurred during the study.

4. **MRID 49741209 "AOAC Use-Dilution Method, Test Organism: Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)" for Frozen, F2015.0012, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – August 11, 2015. Project Number A18321.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%**

SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.4 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 7.40 g of test substance in 1000 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Synthetic broth), mixed and incubated for 24 ± 2 hours at 35-37°C. Then a 10 µL aliquot was transferred to a sufficient number of tubes containing 10 mL of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C, and was vortex mixed (3-4 seconds) and allowed to stand for ≥ 10 minutes before use. The test organism was diluted by adding 8.0 mL of test organism suspension to 24.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. The carriers were dried for 39 minutes at 36.1-36.2°C at 52.6% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. Antibiotic resistance was confirmed using the Kirby Bauer susceptibility assay.

Note: No protocol amendments or deviations occurred during the study.

5. **MRID 49741210 "AOAC Use-Dilution Method, Test Organism: Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NRS 123)" for Frozen, F2015.0012, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – August 11, 2015. Project Number A18322.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Community Associated Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 400 (NRS 123). Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.5 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 1.00 g of test substance in 135.1 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Synthetic Broth), mixed and incubated for 24 ± 2 hours at 35-37°C. A 10 µL aliquot was then transferred to a sufficient number of tubes containing 10 mL of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C, and was vortex mixed (3-4

seconds) and allowed to stand for ≥ 10 minutes before use. The test organism was diluted by adding 15.0 mL of test organism suspension to 15.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. The carriers were dried for 38 minutes at 36°C at 50% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. Antibiotic resistance was confirmed using the Kirby Bauer susceptibility assay.

Note: No protocol amendments or deviations occurred during the study.

6. **MRID 49741211 "AOAC Use-Dilution Method, Test Organism: *Escherichia coli* O157:H7 (ATCC 35150)" for Frozen, F2015.0012, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date – August 11, 2015. Project Number A18323.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.UD.6 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 7.40 g of test substance in 1000 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium (Synthetic broth), mixed and incubated for 24 ± 2 hours at 35-37°C. A 10 µL aliquot was then transferred to sufficient tubes containing 10 mL of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C, and was vortex mixed (3-4 seconds) and allowed to stand for ≥10 minutes before use. The test organism was diluted by adding 15.0 mL of test organism suspension to 15.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. The carriers were dried for 39 minutes at 36.2-36.3°C at 55.3% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 21.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate. All subcultures were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: No protocol amendments or deviations occurred during the study.

7. **MRID 49741212 "Virucidal Hard-Surface Efficacy Test – Enterovirus EV-D68" for Frozen, F2015.0012, by Salimatu Lukula. Study conducted at MircoBioTest. Study completion date – August 10, 2015. Project Number 320-591.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD02 was reported to be **14.12%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Enterovirus EV-D68 (ATCC VR-561) using Vero cells (ATCC CCL-81) as the host system. Two batches (Batch Nos. 15HLD01 and 15HLD02) of the product, Frozen, F2015.0012, were tested according to MicroBioTest Protocol No. 320.2.04.06.15 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 0.74 g of test substance in 100 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 2 hours of preparation. The stock virus culture contained 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at 20-21°C at 29.9-30.1% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 10 minutes at 20°C and 29.8-30.0% relative humidity. The virus-disinfectant mixtures were neutralized using 2.0 mL of 1X Minimum Essential Medium (MEM) + 1% Newborn Calf Serum (NCS) + 0.5% Poly-80 + 0.5% $Na_2S_2O_3$ + 1% $NaHCO_3$ and the plates were scraped with a cell scraper to re-suspend the contents. Serial dilutions were made. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.4 mL of each dilution. The cultures were incubated at 34-38°C in a humidified atmosphere of 4-6% CO_2. The cultures were scored periodically for 6-9 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, plate recovery, cytotoxicity, host cell viability, and neutralization. Viral and cytotoxicity titers were calculated by the Spearman-Karber method.

Note: No protocol deviations occurred during the study. Three protocol amendments were made to clarify the carrier preparation procedure and preparation of the test substance. Testing was conducted as described above.

8. **MRID 49741213 "Virucidal Hard-Surface Efficacy Test – MERS-Coronavirus (MERS-CoV)" for Frozen, F2015.0012, by Salimatu Lukula. Study conducted at MircoBioTest. Study completion date – August 10, 2015. Project Number 320-592.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD02 was reported to be **14.12%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against MERS-Coronavirus (MERS-CoV) (BEI Resources) using Vero E6 cells (ATCC CRL-1586) as the host system. Two batches (Batch Nos. 15HLD01 and 15HLD02) of the product, Frozen, F2015.0012, were tested according to MicroBioTest Protocol

No. 320.3.04.06.15 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 0.74 g of test substance in 100 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 2 hours of preparation. The stock virus culture contained 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at 21°C at 31.9% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 10 minutes at 21°C and 31.9% relative humidity. The virus-disinfectant mixtures were neutralized using 2 mL of 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate-80 + 0.5% $Na_2S_2O_3$ + 1% $NaHCO_3$ and the plates were scraped with a cell scraper to re-suspend the contents. Serial dilutions were made. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.4 mL of each dilution. The cultures were incubated at 34-38°C in a humidified atmosphere of 4-6% CO_2. The cultures were scored periodically for 4-9 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, plate recovery, cytotoxicity, host cell viability, and neutralization. Viral and cytotoxicity titers were calculated by the Spearman-Karber method.

Note: No protocol deviations occurred during the study. Three protocol amendments were made to clarify the carrier preparation procedure and preparation of the test substance. Testing was conducted as described above.

9. **MRID 49741214 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus" for Frozen, F2015.0012, by Mary J. Miller. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number A18338.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Influenza A virus (ATCC VR-544, Strain Hong Kong) using MDCK cells (canine kidney cells; ATCC CCL-34) as the host system. Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18011615.FLUA (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 2.00 g of test substance in 270.3 mL of 100 ppm AOAC synthetic hard water. Following a sponsor-requested SDIC titration, additional hard water was added to dilute the test substance to the lower certified limit. Prepared test substance was used on the day of preparation. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 10 minutes at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the

contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the Spearman-Karber method.

Note: No protocol amendments or deviations occurred during the study.

10. MRID 49741215 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus" for Frozen, F2015.0012, by Mary J. Miller. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number 18340.

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Feline Calicivirus (ATCC VR-782, Strain F-9) as a surrogate virus for Norovirus using CRFK (feline kidney cells, ATCC CCL-94) as the host system. Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18041615.FCAL (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 2.00 g of test substance in 270.3 mL of 100 ppm AOAC synthetic hard water. Following a sponsor-requested SDIC titration, additional hard water was added to dilute the test substance to the lower certified limit. Prepared test substance was used on the day of preparation. On the day of use, two aliquots of stock virus were thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, two dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 10 minutes at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the Spearman-Karber method. The $\log_{10}$ reduction in infectivity was calculated using a Most Probable Number (MPN) statistical method.

Note: No protocol amendments or deviations occurred during the study.

11. **MRID 49741216 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37" for Frozen, F2015.0012, by Mary J. Miller. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 28, 2015. Project Number A18339.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against Rhinovirus type 37 (ATCC VR-1147, Strain 151-1) using MRC-5 cells (human embryonic lung cells; ATCC CCL-171) as the host system. Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18011615.R37 (copy provided). The product was received as a granulated solid. A use solution was prepared for both batches by dissolving 2.00 g of test substance in 270.3 mL of 100 ppm AOAC synthetic hard water. Following a sponsor-requested SDIC titration, additional hard water was added to dilute the test substance to the lower certified limit. Prepared test substance was used on the day of preparation. On the day of use, an aliquot of stock virus was thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 15.5°C at 50% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 10 minutes at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the Spearman-Karber method.

Note: No protocol amendments or deviations occurred during the study.

12. **MRID 49741217 "Fungicidal Use-Dilution Method, Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)" for Frozen, F2015.0012, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 10, 2015. Project Number A18317.**

The active ingredient concentration of Batch No. 15HLD02 was reported to be **14.12%** sodium dichloroisocyanurate dihydrate (SDIC) and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two batches (Batch Nos. 15HLD02 and 15HLD03) of the product, Frozen, F2015.0012, were tested using Accuratus Lab Services Protocol No. CX18012615.FUD.2 (copy provided). The product was

received as a granulated solid. A use solution was prepared for both batches by dissolving 7.40 g of test substance in 1000 mL of 100 ppm AOAC synthetic hard water. Prepared test substance was used within 3 hours of preparation. The test culture was prepared by inoculating 30 Sabouraud Dextrose agar plates using a stock culture and incubating at 25-30°C for 10 days. The mycelia were removed from the plates, and harvested using glass beads in saline/Triton Solution. The culture was then filtered, diluted and stored for 3 days at 2-8°C. The conidial count was 3.43×10^8 conidia/mL. The test organism was diluted by adding 2.00 mL of test organism suspension to 28.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture for 15 minutes. The carriers were dried for 38 minutes at 35.9-36.1°C at 56.1% relative humidity. Carriers were used in the test within 2 hours of drying. Each carrier was placed into 10 mL of test substance for 10 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin + 0.5% Tween 80. Secondary neutralization was performed within 25-60 minutes by transferring the carrier into new individual tubes containing 10 mL of the neutralizer medium (same as primary medium). All subcultures were incubated for 10 days at 25-30°C. The agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: No protocol amendments or deviations occurred during the study.

13. **MRID 49741218 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organism: *Enterobacter aerogenes* (ATCC 13048)" for Frozen, F2015.0012, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 25, 2015. Project Number A18358.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC), Batch No. 15HLD02 was reported to be **14.12%** SDIC, and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). Three batches (Batch Nos. 15HLD01, 15HLD02, and 15HLD03) of the product, Frozen, F2015.0012, were tested according to Accuratus Lab Services protocol CX18012615.NFS.2 (copy provided). The product was received as a granulated solid. A use solution of each batch was prepared by dissolving 7.40 g of Batch 15HLD01 in 1000 mL of 100 ppm AOAC synthetic hard water. Use solutions of Batch 15HLD02 and 15HLD03 were prepared by dissolving 2.00 g of test substance in 270.3 mL of 100 ppm AOAC synthetic hard water. An SDIC titration was performed and additional hard water was added to dilute the test material to the desired concentration. Prepared test substance was used within 3 hours of preparation. From a stock slant ≤5 transfers from the original stock (and ≤1 month old), an initial tube of 10 mL of culture broth (Tryptic Soy Broth) was inoculated. From this initial broth suspension, at least 3 daily transfers of 10 µL were made into 10 mL of culture media. Each daily transfer was incubated for 24 ± 2 hours. The final test culture was incubated for 48-54 hours, was vortex mixed and allowed to stand for ≥15 minutes before use. The test culture was diluted by combining 1.00 mL of organism suspension with 9.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a

5% organic soil load. Five sterile glass carriers per product lot per microorganism were inoculated with 20 µL of suspension of microorganisms using a pipettor. The inoculum was spread to within 3mm of the edges of the carrier. The carriers were dried in a humidity chamber for 22 minutes at 36.0°C at 40% relative humidity. Carriers were transferred to individual sterile plastic jars, and 5.0 mL of test substance was added to the jar. Carriers were submerged for 5 minutes at room temperature (20°C) and 56% relative humidity. After the exposure time, 20.0 mL of neutralizer containing Letheen Broth and 0.07% Lecithin and 0.5% Tween 80 + 0.1% Sodium Thiosulfate was added to the jars and mixed. Within 30 minutes of neutralization, duplicate1.00 and 0.100 mL aliquots of the neutralized solution were plated onto recovery agar plates and incubated for 48 ± 4 hours at 25-32°C. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, carrier quantitation, inoculum count, and neutralization confirmation.

Note: No protocol amendments or deviations occurred during the study.

14. **MRID 49741219 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable), Test Organism: *Staphylococcus aureus* (ATCC 6538)" for Frozen, F2015.0012, by Gracia Schroeder. Study conducted at Accuratus Lab Services. Study completion date (amended) – September 25, 2015. Project Number A18357.**

The active ingredient concentration of Batch No. 15HLD01 was reported to be **14.59%** sodium dichloroisocyanurate dihydrate (SDIC), Batch No. 15HLD02 was reported to be **14.12%** SDIC, and Batch No. 15HLD03 was reported to be **13.96%** SDIC. The use solutions were prepared and diluted to simulate product at or below the Lower Certified Limit. All batches meet EPA's criteria for efficacy testing, as detailed in the Agency's guidance document "Lower Certified Limit Testing Guidance" (12/6/2013).

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three batches (Batch Nos. 15HLD01, 15HLD02, and 15HLD03) of the product, Frozen, F2015.0012, were tested according to Accuratus Lab Services protocol CX18012615.NFS.1 (copy provided). The product was received as a granulated solid. A use solution of each batch was prepared by dissolving 7.40 g of Batch 15HLD01 in 1000 mL of 100 ppm AOAC synthetic hard water. Use solutions of Batch 15HLD02 and 15HLD03 were prepared by dissolving 2.00 g of test substance in 270.3 mL of 100 ppm AOAC synthetic hard water. An SDIC titration was performed and additional hard water was added to dilute the test material to the desired concentration. Prepared test substance was used within 3 hours of preparation. From a stock slant ≤5 transfers from the original stock (and ≤1 month old), an initial tube of 10 mL of culture broth (Nutrient Broth) was inoculated. From this initial broth suspension, at least 3 daily transfers of 10 µL were made into 10 mL of culture media. Each daily transfer was incubated for 24±2 hours. The final test culture was incubated for 48-54 hours, vortex mixed, and allowed to stand for ≥15 minutes before use. The test culture was diluted in sterile growth medium by combining 3.00 mL of organism suspension with 3.0 mL of sterile growth medium. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five sterile glass carriers per product lot per microorganism were inoculated with 20 µL of suspension of microorganisms using a pipettor. The inoculum was spread to within 3mm of the edges of the carrier. The carriers were dried in a humidity chamber for 20 minutes at 35.9°C at 40% relative humidity. Carriers were transferred to individual sterile plastic jars, and 5.0 mL of test substance was added to the jar. Carriers were submerged for 5 minutes at room temperature (20°C) and 53% relative humidity. After the exposure time, 20.0 mL of neutralizer containing Letheen Broth and 0.07% Lecithin and 0.5% Tween 80 + 0.1% Sodium Thiosulfate was added to the jars and mixed. Within 30 minutes of neutralization,

duplicate 1.00 and 0.100 mL aliquots of the neutralized solution were plated onto recovery agar plates and incubated for 48 ± 4 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, carrier quantitation, inoculum count, and neutralization confirmation.

Note: No protocol amendments or deviations occurred during the study.

V RESULTS

Disinfection – Bactericidal Efficacy

MRID	Organism	Test Date	No. Carriers Exhibiting Growth/Total Carriers			Carrier Population (Log_{10} CFU/Carrier)
			Batch 15HLD01	**Batch 15HLD02**	**Batch 15HLD03**	
10 minute contact time, 100 ppm hard water, 5% organic soil						
49741206	*Staphylococcus aureus* (ATCC 6538)	4/30/15	1/60*	--	--	6.37
		5/8/15	1/60	--	--	6.36
		5/4/15	--	1/60	--	6.35
		5/5/15	--	--	0/60	6.46
49741207	*Pseudomonas aeruginosa* (ATCC 15442)	4/30/15	0/60*	--	--	6.00
		5/6/15	1/60	--	--	6.59
		5/4/15	--	1/60	--	6.59
		5/5/15	--	--	0/60	5.76**
		5/20/15	--	--	0/60	6.16
49741208	*Salmonella enterica* (ATCC 10708)	4/30/15	0/60	0/60	0/60	5.65
49741209	Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592)	5/4/15	--	0/10	0/10	6.01
49741210	Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NRS 123)	5/4/15	--	0/10	0/10	5.76
49741211	*Escherichia coli* O157:H7 (ATCC 35150)	5/4/15	--	0/10	0/10	5.98

*Soil load <5%, testing repeated

**Carrier population control failure, testing repeated

Disinfection – Virucidal Efficacy

MRID	Organism	Description	Batch 15HLD01	Batch 15HLD02	Batch 15HLD03	Dried Virus Control ($TCID_{50}$)
10 minute contact time, 100 ppm hard water, 5% organic soil						
49741212	Enterovirus EV-D68 (ATCC VR-561)	10^{-2} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	--	$10^{5.85}$
		$TCID_{50}$/0.4mL	$\leq 10^{1.10}$	$\leq 10^{1.10}$		
		Log Reduction	≥4.75	≥4.75		
49741213	MERS-Coronavirus (MERS-CoV) (BEI Resources)	10^{-3} to 10^{-7} Dilutions	Complete Inactivation	Complete Inactivation	--	$10^{6.10}$
		$TCID_{50}$/0.4mL	$\leq 10^{1.10}$	$\leq 10^{1.10}$		
		Log Reduction	≥5.00	≥5.00		
49741214	Influenza A Virus, Strain Hong Kong (ATCC VR-544)	10^{-1} to 10^{-8} dilutions	--	Complete Inactivation	Complete Inactivation	$10^{5.50}$
		$TCID_{50}$/0.1mL		$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		TCD_{50}/0.1mL		$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction		≥5.00	≥5.00	
49741215	Feline Calicivirus (Norovirus surrogate) Strain F-9 (ATCC VR-782)	10^{-1} to 10^{-4} dilutions	--	Complete Inactivation	Complete Inactivation	$10^{6.25}$* (Log_{10} MPN = 6.05894)
		$TCID_{50}$/0.1mL		$\leq 10^{0.50}$*	$\leq 10^{0.50}$*	
		TCD_{50}/0.1mL		$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction		≥6.06	≥6.06	
49741216	Rhinovirus type 37 Strain 151-1 (ATCC VR-1147)	10^{-1} to 10^{-6} dilutions	--	Complete Inactivation	Complete Inactivation	$10^{6.00}$
		$TCID_{50}$/0.1mL		$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		TCD_{50}/0.1mL		$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction		≥5.50	≥5.50	

*Same result for both surface replicates

Disinfection – Fungicidal Efficacy

MRID	Organism	Test Date	No. Carriers Exhibiting Growth/Total Carriers			Carrier Population (Log_{10} CFU/Carrier)
			Batch 15HLD01	Batch 15HLD02	Batch 15HLD03	
10 minute contact time, 100 ppm hard water, 5% organic soil						
49741217	*Trichophyton mentagrophytes*	5/4/15	--	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	4.89

	(ATCC 9533)					

Non-Food Contact Surface Sanitizing Efficacy

MRID	Organism	Batch	Test Result (log_{10} geometric mean CFU/carrier)	Carrier Population (log_{10} geometric mean CFU/carrier)	Percent Reduction
5 minute contact time, 400 ppm hard water, 5% organic soil					
49741218	*Enterobacter aerogenes* (ATCC 13048)	15HLD01	<1.40	6.03	>99.9%
		15HLD02	<1.40		>99.9%
		15HLD03	<1.40		>99.9%
49741219	*Staphylococcus aureus* (ATCC 6538)	15HLD01	<1.40	6.40	>99.9%
		15HLD02	<1.40		>99.9%
		15HLD03	<1.40		>99.9%

VI CONCLUSIONS

1. The submitted efficacy data **support** use of the product, Frozen, F2015.0012, as a disinfectant with bactericidal activity on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10 minute contact time when diluted at 7.4g/L 100 ppm hard water against the following bacteria:

   Staphylococcus aureus (ATCC 6538) MRID 49741206
   Pseudomonas aeruginosa (ATCC 15442) MRID 49741207
   Salmonella enterica (ATCC 10708) MRID 49741208
   Methicillin Resistant *Staphylococcus aureus* – MRSA (ATCC 33592) MRID 49741209
   Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NRS 123) MRID 49741210
   Escherichia coli O157:H7 (ATCC 35150) MRID 49741211

   According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data **support** the use of the product, Frozen, F2015.0012, as a disinfectant with virucidal activity on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10 minute contact time when diluted at 7.4g/L 100 ppm hard water against the following viruses:

   Enterovirus EV-D68 (ATCC VR-561) MRID 49741212

MERS-Coronavirus (MERS-CoV), (BEI Resources) MRID 49741213
Influenza A Virus, Strain Hong Kong (ATCC VR-544) MRID 49741214
Feline Calicivirus (Norovirus surrogate), Strain F-9 (ATCC VR-782) MRID 49741215
Rhinovirus type 37, Strain 151-1 (ATCC VR-1147) MRID 49741216

According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested.

3. The submitted efficacy data **support** the use of the product, Frozen, F2015.0012, as a disinfectant with fungicidal activity on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10 minute contact time when diluted at 7.4g/L 100 ppm hard water against the following fungus:

Trichophyton mentagrophytes (ATCC 9533) MRID 49741217

According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

4. The submitted efficacy data **support** the use of the product, Frozen, F2015.0012, as a sanitizer on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 5 minute contact time when diluted at 7.4g/L 100 ppm hard water against the following bacteria:

Enterobacter aerogenes (ATCC 13048) MRID 49741218
Staphylococcus aureus (ATCC 6538) MRID 49741219

According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VII LABEL

1. The proposed label claims that the product, Frozen, is a disinfectant against the following bacteria on hard, non-porous surfaces for a 10 minute contact time in the presence of 5% organic soil when diluted at 2 tbsp/gallon (7.4g/L) water:

Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)
Pseudomonas aeruginosa (ATCC 15442)
Methicillin Resistant *Staphylococcus aureus* – MRSA (ATCC 33592)
Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype

USA 400 (NRS 123)
Escherichia coli O157:H7 (ATCC 35150)

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Frozen, is a disinfectant with fungicidal activity against the following fungus on hard, nonporous surfaces for a 10 minute contact time in the presence of 5% organic soil when diluted at 2 tbsp/gallon (7.4g/L) water:

   Trichophyton mentagrophytes (ATCC 9533)

   This claim is acceptable as it is supported by the submitted data.

3. The proposed label claims that the product, Frozen, is a disinfectant with virucidal activity against the following viruses on hard, nonporous surfaces for a 10 minute contact time in the presence of 5% organic soil when diluted at 2 tbsp/gallon (7.4g/L) water:

   Rhinovirus type 37, Strain 151-1 (ATCC VR-1147)
   Feline Calicivirus (Norovirus surrogate), Strain F-9 (ATCC VR-782)
   Influenza A Virus, Strain Hong Kong (ATCC VR-544)
   MERS-Coronavirus (MERS-CoV), (BEI Resources)
   Enterovirus EV-D68 (ATCC VR-561)

   These claims are acceptable as they are supported by the submitted data.

4. The proposed label claims that the product, Frozen, is a sanitizer against the following bacteria on hard, non-porous, non-food contact surfaces for a 5 minute contact time in the presence of 5% organic soil when diluted at 2 tbsp/gallon (7.4g/L) water:

   Enterobacter aerogenes (ATCC 13048)
   Staphylococcus aureus (ATCC 6538)

   These claims are acceptable as they are supported by the submitted data.

5. On page 4 of the proposed label, in the directions for use for sanitizing, the heading should be changed to “[For] Sanitizing –or- To Sanitize Hard, Non-porous, Non-food Contact Surfaces.” The phrase “[For surfaces that may come in contact with food…]” should be removed. The product is not approved as a food contact surface sanitizer.

6. On page 4 of the proposed label, in the directions for use for sanitizing, the contact time for “Work Surfaces” may be changed to 5 minutes.

7. On pages 5, 6, 11, and 13 of the proposed label, the directions for use and marketing claims that refer to treating “mold and mildew stains” should be limited to treatments “for aesthetic purposes.”

8. On page 6 of the proposed label, the directions for “Household Use” direct the user to apply the product to hard, non-porous surfaces. The directive is followed by directions for “Laundry Use.” This may be misleading as the product can disinfect and sanitize hard, non-porous surfaces, but may only be used for bleaching or cleaning in laundry.

9. On page 8 of the proposed label, the strain number for Community Associated Methicillin Resistant *Staphylococcus aureus* should be changed to " …[(Genotype USA 400)]."

10. On page 8 of the proposed label, the strain number for *Escherichia coli* O157:H7 should be changed to "[ATCC 35150]."

11. On page 8 of the proposed label, the phrase "[(a common cause of food-borne illness)]" associated with *Salmonella enterica* should be removed.

12. On page 8 of the proposed label, the phrase "[(a common cause of Staph infection)]" associated with *Staphylococcus aureus* should be removed.

13. On page 9 of the proposed label, the outdoor use surfaces "Bike –or- bicycle", "Outdoor siding", "Playground sets", and "Sides of house" should be qualified the "◊◊◊hard, nonporous surfaces of" qualifier.

14. On page 10 of the proposed label, the phrase "hard non-porous surfaces" should be added to the use site "[All] Around the House."

15. On page 11 of the proposed label, the 10th bullet in the 1st column should be qualified with "when applied according to the directions for disinfection" or a similar statement.

16. On pages 11 and 12 of the proposed label, the footnote "§§" associated with "germs" is unnecessary because organisms from the bacteria, viruses, and fungi are included on the label. The qualifier may be confusing because all of the organisms on the label may be considered "germs", not only the 4 listed organisms.

17. On page 11 of the proposed label, in the 3rd bullet from the end of the 1st column, "[around the house]" should be limited to "on hard non-porous surfaces."

18. On page 11 of the proposed label, the claims in the 3rd bullet of the 2nd column and in the 3rd bullet from the end of the 3rd column should be removed. Efficacy data was not submitted or cited to support emergency drinking water disinfection.

19. On page 11 of the proposed label, in the 4th bullet of the 2nd column, "home" should be limited to "hard non-porous surfaces."

20. On page 11 of the proposed label, the claim in the 4th bullet of the 3rd column should be removed. The product was not tested as a laundry sanitizer or disinfectant.

21. On page 11 of the proposed label, in the 9th bullet of the 3rd column, "surface" should be limited to "hard, non-porous surfaces."

22. On page 11 of the proposed label, in the 11th bullet of the 3rd column, "on hard, non-porous surfaces" should be added before "around your home..."

23. On page 11 of the proposed label, in the 13th bullet of the 3rd column, the claim appears incomplete. A reference to the surfaces in List 3 would be acceptable to end the claim.

24. On page 11 of the proposed label, in the 14th bullet of the 3rd column, "that can cause food-borne illness §§§" and the footnote "§§§" should be removed.

25. On page 12 of the proposed label, in the 4th bullet of the 1st column, “home” should be limited to “hard non-porous surfaces” and “kitchen” should be limited to “hard, non-porous, non-food contact surfaces.”

26. On pages 16-18 of the proposed label, the service bulletins for emergency disinfection of drinking water and use in spas, hot tubs, and immersion tanks should be removed. Data supporting these uses should be submitted or cited according to the guidelines in OCSPP 810.2600.